98-272943/25 B04 C07 D16 E12 J04 (E24) GLUE/ 96.11.06

GLUESENKAMP K *DE 19645601-A1

96.11.06 96DE-1045601 *(98.05.07)* C07F 5/04, A01N 55/08, C09B 69/10, C09K 11/06, A61K 31/69

New bio-specific compound boronic acid derivatives - useful for labelling carriers, e.g. cells or tissues, with probes under mild conditions,

C98-085228

Addnl. Data: GLUESENKAMP K, KOSEGARTEN H, STEINWEG D

BC(5-B1A) D(5-A1, 5-H9) E(5-C2) J(4-B1) .1

Boronic acid derivatives of formula (I) and (II) are new.

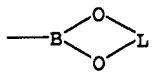
$(Z)_kY\text{-}B(OH)_2$ (I) $[(Z)_k\text{-}Y\text{-}B]_mC'$ (II)

Y = optionally substituted, optionally unsaturated alkylidene (preferably propylidene or 2-methyltrimethylene) or substituted cyclic residue of a saturated, heterocyclic, alicyclic or aromatic (such as 1,3-phenylene or benzylene);

Z = optionally substituted biospecific compound (A), preferably with one or more residues of formula -D-E coupled to it;

D = substituted group as for Y;

E = $B(OH)_2$, $OB(OH)_2$ or group of formula (a);



(a)

L = T or Z';

Z' = Z having vicinal substituted or unsubstituted OH groups;

T = (preferably polymeric) carrier residue with vicinal substituted and/or substituted OH groups;

C' = -O-T-O-;

k, m = 1-10.

USE

(I) and (II) are biologically, biochemically, pharmaceutically or diagnostically active compounds (A), which are enzyme inhibitors, herbicides or pesticides, antibiotics or antimycotics, or (for (II) only) dyes (especially substituted fluoresceins) (all claimed). (I) and (II) are useful for labelling polymeric carriers T, which are plant cells, cell organelles, parts of these, tissues or tissue slices (all claimed), with (A). The plant cell membranes can be specifically labelled with probes

for the study of membrane properties such as pH and ion flux, e.g. Fe^{2+}/Fe^{3+} transport.

ADVANTAGE

Carriers can be selectively labelled under mild conditions without affecting the activity of (A). The adducts contain cyclic borate ester bonds which are very stable under physiological conditions.

PREPARATION

Claimed preparation of (I) involves:

(a) incubating (A) for 1-5 hours with a substituted boronic acid derivative (III), preferably at room temperature, in presence of a polar solvent and a base (preferably triethylamine); and

(b) adding ether to the mixture to precipitate (I), and drying.

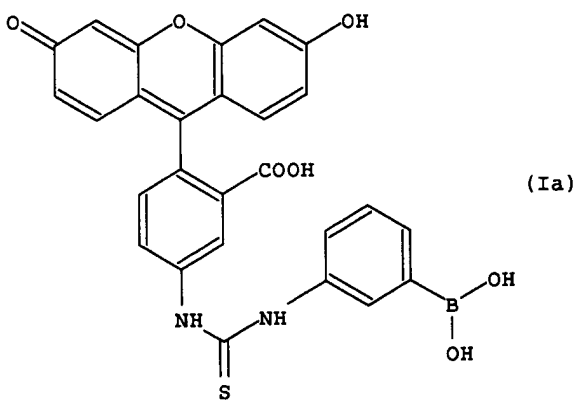
Claimed preparation of (II) comprises preparing (I) as above, and esterifying with carrier T, preferably at room temperature, at pH 6-8 (preferably 7).

EXAMPLE

A stirred mixture of 0.173g aminobenzene-boronic acid hydrochloride, 0.389g fluorescein isothiocyanate (FITC) and 20 ml DMF was treated with 1 equivalent of NEt_3. After 5 hours, the product was precipitated in ether, washed with ether and dried to give 0.5g (93%) of the adduct of formula (Ia).

Cultured wheat root cell samples were incubated for 1-12 hours in the dark with various concentrations of (Ia) or non-derivatised FITC (10-100 mM in PBS). After washing, the samples were compared using a confocal laser microscope (excitation 490 nm, fluorescence 600 nm). Samples incubated with (Ia) showed a stable fluorescence signal, whereas those incubated with FITC did not. (LJ)

98-272943/25



(Ia)

(8pp2400DwgNo.0/1)

New bio-specific compound boronic acid derivatives

Patent Number: DE19645601
Publication date: 1998-05-07
Inventor(s): GLUESENKAMP KARL-HEINZ DR (DE); STEINWEG DETLEF (DE); KOSEGARTEN HARALD DR (DE)
Applicant(s): GLUESENKAMP KARL HEINZ DR (DE)
Requested Patent: DE19645601
Application Number: DE19961045601 19961106
Priority Number(s): DE19961045601 19961106
IPC Classification: C07F5/04; A01N55/08; C09B69/10; C09K11/06; A61K31/69; C08B37/00; C08B15/05; G01N1/30; C12Q1/00
EC Classification: C07F5/02C, G01N33/532, G01N33/58
Equivalents:

Abstract

Boronic acid derivatives of formula (Z)kY-B(OH)2 (I) and (Z)k-Y-BmC' (II) are new. Y = optionally substituted, optionally unsaturated alkylidene (preferably propylidene or 2-methyltrimethylene) or substituted cyclic residue of a saturated, heterocyclic, alicyclic or aromatic (such as 1,3-phenylene or benzylene); Z = optionally substituted biospecific compound (A), preferably with one or more residues of formula -D-E coupled to it; D = substituted group as for Y; E = B(OH)2, OB(OH)2 or group of formula (a); L = T or Z'; Z' = Z having vicinal substituted or unsubstituted OH groups; T = (preferably polymeric) carrier residue with vicinal substituted and/or substituted OH groups; C' = -O-T-O-; k, m = 1-10.